THE MOLECULAR WEIGHT AND DISSOCIATION PROPERTIES OF YEAST HEXOKINASE

J.S.EASTERBY and M.A.ROSEMEYER
Department of Biochemistry, University College London, London, U.K.

Received 19 June 1969

Hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) is the first enzyme of the glycolytic pathway and as such is of critical importance to the overall energy-producing metabolism of the cell. Its key position in glycolysis also suggests that it may be subject to metabolic control by allosteric effectors. Before such phenomena can be assessed it is necessary to determine the number of subunits of the enzyme and to investigate their interactions. The present research attempts to establish the molecular weight of the enzyme and to characterise the various dissociation equilibria in which it is involved. It also shows that, owing to the dissociation phenomena, discrete molecular species were seldom observed.

Hexokinase was purified from yeast by Berger, Slein, Colowick and Cori [1] in 1946, and in the same year by Kunitz and McDonald [2]. The latter authors were the first to estimate the molecular weight as 96,600 in acetate at pH 5.5. Subsequent preparations have provided evidence that multiple forms of the enzyme occur [3]. Some of these forms appear to be produced by proteolytic modification of the native hexokinases, and differ from them in molecular and electrophoretic properties. Recently Lazarus, Derechin and Barnard [4] have reported molecular weights of 102,000 and 104,000 in acetate buffer, pH 5.5 *I* 0.1.

Hexokinase was isolated from baker's yeast (Easterby and Rosemeyer, unpublished work). Each of the proteins obtained was homogeneous on starch gel electrophoresis and ultracentrifugation. During the purification, addition of phenylmethyl sulphonylfluoride (PMSF) to protect against proteolysis [5] resulted in two forms of the enzyme, designated hexokinases A and B. These hexokinases had specific activities of 200 and 600 units/mg respectively, defining a unit according to Darrow and Colowick [6]. The proteins appear to be similar to the forms P-I and P-II prepared by Gazith et al. [3]. In the absence of PMSF two modified forms of the enzyme were obtained, termed A′ and B′, which had specific activities of 300 and 600 units/mg respectively.

The modified form, A′, was found to dissociate reversibly between pH 5.0 and pH 7.0. In phosphate buffer pH 7.0 *I* 0.1 a species with a sedimentation coefficient of 3.85 S was observed (fig. 1). In the same solvent equilibrium ultracentrifugation, according to the method of Van Holde and Baldwin [7], gave a molecular weight of 55,500 for the A′ enzyme. A value of 0.74 was used for $\bar{V}$ from Kenkare and Colowick [8]. The weight-average (M_w) and *z*-average (M_z) molecular weights were the same under these conditions, and M_w remained constant through the solution column, suggesting that a discrete species (the half-molecule) was present. Extensive association of the A′ enzyme towards the full molecule occurred in acetate buffer, pH 5.0 *I* 0.1. In this solvent, the sedimentation coefficient was 5.78 S, M_w was 98,300 and M_z 104,000. The disparity between M_w and M_z indicates that association was incomplete. This conclusion was supported by an increase of M_w through the solution column. According to sedimentation coefficients calculated for the discrete species as outlined below, a plateau was reached at low pH when the weight-average degree of dissociation was 0.23 (fig. 1).

The native A and B enzymes did not show a sharp pH-dependent dissociation. However, dissociation was promoted by increasing ionic strength, and was also

North-Holland Publishing Company – Amsterdam

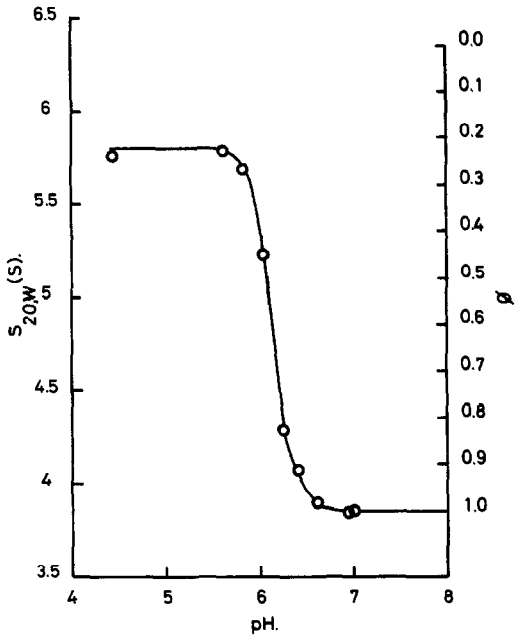

Fig. 1. Variation of sedimentation coefficient and estimated degree of dissociation (ϕ) of hexokinase A′ with pH. All solutions were in phosphate I 0.1. The temperature was 10° and the protein concentration 2.5 mg/ml.

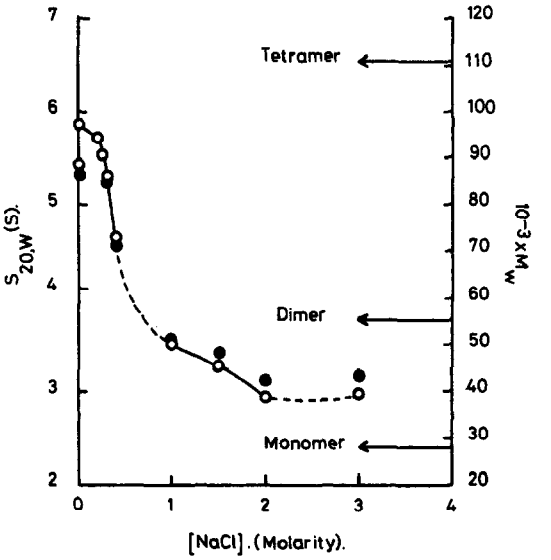

Fig. 2. Variation of sedimentation coefficient (○) and molecular weight (●) of hexokinase A with salt concentration. The buffer was phosphate pH 7.0 I 0.1

enhanced by the presence of substrate hexoses. In acetate buffer pH 5.0 I 0.1 hexokinase A behaved like the A′ enzyme, with an $S_{20,w}$ of 5.76 S. The M_w was 96,000 and M_z 104,500. The discrepancy again indicates incomplete association to the full molecule. On lowering the ionic strength, further association occurred. In phosphate pH 7.0, with the ionic strength 0.1, the extent of dissociation was small in comparison with that observed with the A′ enzyme. At neutral pH, when the ionic strength was greater than 0.9, the molecular weight observed for hexokinase A was less than suggested above for the half-molecule (fig. 2), showing that further dissociation of this species occurred.

From the dissociation equilibria of the native and modified forms of hexokinase, the molecular weights of the discrete species may be deduced. Thus according to Sophianopoulos and Van Holde [9] the values for M_w and M_z in a monomer-polymer equilibrium are related by:

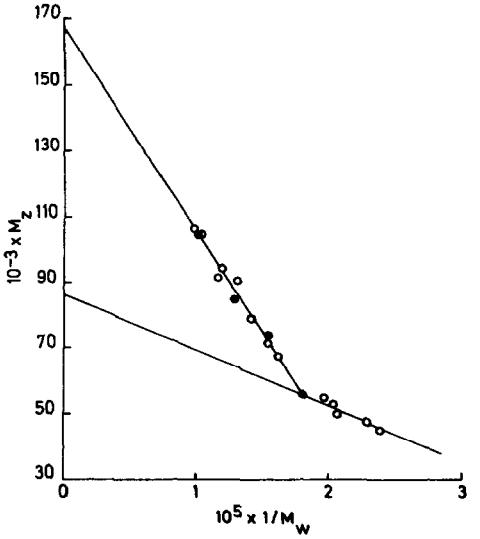

Fig. 3. Relation between the molecular weights M_w and M_z for hexokinase A (○) and for hexokinase A′ (●).

$$M_z = (n+1)M_1 - nM_1^2/M_w \ .$$

A plot of M_z against the reciprocal of M_w should give

a straight line, and from the intercept and slope the monomer molecular weight M_1 and the degree of polymerisation n may be determined. Fig. 3 shows this plot for the A and A′ enzymes. The data from both proteins show similar behaviour. Moreover, the results on hexokinase A fall on two distinct lines indicating two separate dissociation steps. The second of these equilibria occurred at high salt concentrations (fig. 2) and represents the dissociation of the half-molecule to the quarter-molecule. From the values obtained for the molecular weights of the discrete species the dissociations may be represented as:

$$\underset{111{,}000}{P_4} \rightleftharpoons \underset{55{,}500}{2P_2} \rightleftharpoons \underset{28{,}000}{4P} \quad ,$$

according to which the full molecule is a tetramer of 111,000 molecular weight.

It is useful to determine the sedimentation coefficients corresponding to the discrete species to enable the degree of dissociation to be assessed from sedimentation data (fig. 1). Thus the weight-average sedimentation coefficient $\bar{S}$ is related to M_w by:

$$\bar{S} = S_n - (M_w/M_1 - n)(S_n - S_1)/(1 - n)$$

in which S_n and S_1 are the sedimentation coefficients of the polymer and monomer taking part in the dissociation equilibrium. A more satisfactory correlation, in that it uses more of the available data, is:

$$S = S_n - (M_w M_z/M_1^2 - n^2)(S_n - S_1)/(1 - n^2) \, .$$

Plots of $\bar{S}$ against M_w, using the former equation, or against $M_w M_z$ using the latter, should be linear. Having determined the value of n for each dissociation above (namely 2), the sedimentation coefficients for the individual species may be obtained:

Hexokinase A: tetramer 6.4 S, dimer 3.6 S, monomer 2.2 S.

Hexokinase A′: tetramer 6.35 S, dimer 3.85 S.

The above analysis of the equilibria indicates that at pH 5.0 both the A and A′ forms of hexokinase exist predominantly as tetramers of 111,000 molecular weight in reversible dissociation equilibrium with dimers of 55,500 molecular weight. In high concentrations of NaCl the native enzyme undergoes further dissociation towards a monomer of 28,000 molecular weight. In low salt concentrations, the A enzyme persists predominantly as a tetramer at pH 7.0, while the A′ enzyme shows complete dissociation to the 55,500 molecular weight dimer. This pH-dependent dissociation may be correlated with the ionisation of two equivalent groups with a pK of 5.8 in the dimer, which are shielded from the solvent on association to the tetramer, the pK being greater than 7.

The analysis of the dissociation equilibria of hexokinase presented here reconciles the discrepancy in previous molecular weight measurements in similar solvent conditions. Thus the value of 96,600 [2] was a weight-average value, while that of 104,000 was a determination of M_z [4]. These results agree with the present observations under the same solvent conditions. However the results here obtained are interpreted as representing the partial dissociation of a protein with a molecular weight of 111,000.

Acknowledgement

We are grateful to the Medical Research Council for a Postgraduate Research Scholarship (to J.S.E.), and for providing a Spinco Model E analytical ultracentrifuge.

References

[1] L.Berger, M.W.Slein, S.P.Colowick and C.F.Cori, J. Gen. Physiol. 29 (1946) 379.
[2] M.Kunitz and M.R.McDonald, J. Gen. Physiol. 29 (1946) 393.
[3] J.Gazith, I.T.Schulze, R.H.Gooding, F.C.Womack and S.P.Colowick, Ann. N.Y. Acad. Sci. 151 (1968) 307.
[4] N.R.Lazarus, M.Derechin and E.A.Barnard, Biochemistry 7 (1968) 2390.
[5] I.T.Schulze, J.Gazith and R.H.Gooding, in: Methods in Enzymology, vol. 9, ed. W.A.Wood (Academic Press, New York, 1967) p. 376.
[6] R.A.Darrow and S.P.Colowick, in: Methods in Enzymology, vol. 5, eds. S.P.Colowick and N.O.Kaplan (Academic Press, New York, 1962) p. 226.
[7] K.E.Van Holde and R.L.Baldwin, J. Phys. Chem. 62 (1958) 734.
[8] U.W.Kenkare and S.P.Colowick, J. Biol. Chem. 240 (1965) 4570.
[9] A.J.Sophianopoulos and K.E.Van Holde, J. Biol. Chem. 239 (1964) 2516.